Molecular Biology of the Cell
Vol. 5, 955–966, September 1994

Nucleologenesis: U3 snRNA-containing Prenucleolar Bodies Move to Sites of Active Pre-rRNA Transcription After Mitosis

Luis F. Jiménez-García,* M. de L. Segura-Valdez,* Robert L. Ochs,† Lawrence I. Rothblum,‡ Ross Hannan,‡ and David L. Spector§‖

*Laboratory of Electron Microscopy, Faculty of Sciences, National Autonomous University of Mexico, Mexico, D.F., Mexico; †Scripps Research Institute, La Jolla, California, 92037; ‡Geisinger Clinic, Weis Center for Research, Danville, Pennsylvania 17822; §Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724

Submitted October 11, 1993; Accepted July 22, 1994
Monitoring Editor: Joseph Gall

We have investigated the distribution of U3 snRNA and rRNA in HeLa cells and normal rat kidney cells during interphase and mitosis. U3 snRNA, known to be involved in pre-rRNA processing, was detected in nucleoli and coiled bodies during interphase, whereas rRNA was distributed in the nucleoli and throughout the cytoplasm. By comparison, ribosomal protein S6 was detected in nucleoli, coiled bodies, and in the cytoplasm. During nucleologenesis, pre-rRNA was observed in newly forming nucleoli during late telophase but not in prenucleolar bodies (PNBs), whereas U3 snRNA was detected in forming nucleoli and PNBs. Similar findings to those reported here for the localization of U3 snRNA have been reported previously for the U3 small nuclear ribonucleoprotein fibrillarin. These results suggest that components involved in pre-rRNA processing localize to discrete PNBs at the end of mitosis. The nucleolus is formed at specific telophase domains (nucleolar organizing regions) and the PNBs, containing factors essential for pre-rRNA processing, are recruited to these sites of rRNA transcription and processing.

INTRODUCTION

The nucleolus is a well defined intranuclear organelle where the synthesis and processing of pre-rRNA and preribosome assembly occurs (for a review see Busch and Smetana, 1970; Hadjiolov, 1985; Scheer and Benavente, 1990). Upon transcription of the tandemly arranged rDNA genes located in the nucleolus of mammalian cells, pre-rRNA molecules (45S) are produced that undergo a series of specific cleavages to give rise to 28S, 5.8S, and 18S rRNAs that are present in mature cytoplasmic ribosomes (Perry, 1976; Hadjiolov, 1985). In this process, U3 small nuclear ribonucleoprotein particles (snRNP) have been implicated in one of the earliest processing steps involved in cleavage of 45S pre-rRNA into mature rRNAs (Kass *et al.*, 1990; Savino and Gerbi, 1990; Hughes and Ares, 1991). Among the nucleolar snRNAs, U3 is the most abundant nucleolar-specific snRNA and is highly conserved among eukaryotes (Reddy and Busch, 1988). U3 snRNA and pre-rRNA have been colocalized to the dense fibrillar component of nucleoli as well as to the granular component (Puvion-Dutilleul *et al.*, 1991, 1992). Several other less abundant nucleolar snRNPs, U8 and U13, have been identified and are also thought to have a role in rRNA processing (Tyc and Steitz, 1989; Peculis and Steitz, 1993).

During prophase of mitosis the nucleolus breaks down, and then it reforms during telophase at specific regions of defined chromosomes termed nucleolar organizers (NORs) (McClintock, 1934). The process of nucleolar reformation has been termed nucleologenesis (De la Torre *et al.*, 1975; Morcillo *et al.*, 1976; De la Torre and Giménez-Martín, 1982; Ochs *et al.*, 1985a; Jiménez-García *et al.*, 1989). Nucleologenesis is characterized by the early appearance of scattered nucleolar bodies, which have been called prenucleolar bodies (PNBs) (Stevens, 1965), in telophase nuclei. These bodies fuse at the NORs in late telophase, thereby

‖ Corresponding author: Dr. David L. Spector, Cold Spring Harbor Laboratory, One Bungtown Road, Cold Spring Harbor, NY 11724

forming the interphase nucleolus (McClintock, 1934; Lafontaine, 1958; Lafontaine and Chaouinard, 1963; Stevens, 1965; Stockert *et al.*, 1970; Morcillo *et al.*, 1976; De la Torre and Giménez-Martín, 1982; Ochs *et al.*, 1985a; Montgomery, 1898). Because there is little to no transcriptional activity during mitosis until telophase, this dynamic process affords a unique opportunity to analyze the relationship between rRNA synthesis and the localization of U3 snRNP. In this context, several studies have attempted to elucidate the events involved in nucleologenesis. First, genetic, morphological and cytochemical investigations have led to the conclusion that PNBs and NORs are composed of RNA and rDNA, respectively (Ritossa and Spiegelman, 1965). More recently, cytochemical and immunocytochemical approaches have revealed that PNBs contain specific nucleolar proteins such as fibrillarin (Ochs *et al.*, 1985a,b; Jiménez-García *et al.*, 1989), B23/NO38 (nucleophosmin) (Ochs *et al.*, 1985b; Schmidt-Zachmann *et al.*, 1987), C23 (nucleolin) (Ochs *et al.*, 1985b; Pfeifle *et al.*, 1986), and the 180-kDa protein from *Xenopus* (Bell *et al.*, 1992), as well as argyrophilic proteins (Ochs *et al.*, 1985a,b; Ploton *et al.*, 1987; Jiménez-García *et al.*, 1989). In addition, Azum-Gelade *et al.* (1994) have localized U3 snRNA in PNBs in Chinese hamster ovary (CHO) cells. During telophase, all of these components fuse in the form of PNBs to the NOR, where rDNA, RNA polymerase I, and the transcription factor upstream building factor (NOR-90) are present throughout mitosis (Scheer and Rose, 1984; Jiménez-García *et al.*, 1989; Chan *et al.*, 1991).

In the present study, we have examined the distribution of rRNA and U3 snRNA during interphase and mitosis. We have found that U3 snRNA is localized in both nucleoli and coiled bodies during interphase, whereas rRNA was distributed in nucleoli and throughout the cytoplasm. During nucleologenesis, pre-rRNA was observed in newly forming nucleoli during late telophase but not in PNBs, whereas U3 snRNA was detected in forming nucleoli and PNBs. These results demonstrate that components involved in pre-rRNA processing localize to discrete PNBs at the end of mitosis and that upon initiation of rRNA transcription the PNBs containing factors essential for pre-rRNA processing are recruited to sites of rRNA transcription.

MATERIALS AND METHODS

Cells

Human HeLa (#2-CCL, American Type Culture Collection, Rockville, MD) and normal rat kidney (NRK-52E) epithelial cells (American Type Culture Collection, CLR 1571) were grown on glass coverslips for 1 or 2 d in Dulbecco's modified Eagle's medium (GIBCO, Grand Island, NY) containing glutamine, pyruvate, and 10% fetal bovine serum (GIBCO).

Silver Staining

Ag-NOR silver staining was conducted as described (Goodpasture and Bloom, 1975).

Antibodies

Rabbit polyclonal antibody R288 (Andrade *et al.*, 1993) and human autoantibody Sh (Andrade *et al.*, 1991) to coilin (p80) have been previously characterized (Andrade *et al.*, 1991). These antibodies were used at a dilution of 1:100. Rabbit polyclonal antibody against a synthetic peptide to ribosomal protein S6 was a gift from Robert Traut (University of California, Davis) and was used at a dilution of 1:200. This antibody was made by immunizing rabbits with synthetic peptide RRRLSSLRASTSKASESSQK representing the carboxy terminal 20 amino acids of S6 (Chan and Wool, 1988). Texas red conjugated secondary antibodies (Organon Teknika, Malvern, PA) were used at a dilution of 1:50 in phosphate-buffered saline (PBS).

Immunoblotting

Gel electrophoresis and Western blotting were done according to the procedure of Chan and Pollard (1992). Twenty-five micrograms of protein was loaded in each lane from a HeLa whole cell extract, mixed with electrophoresis sample buffer (63 mM tris(hydroxymethyl)-aminomethane-HCl, 10% glycerol, 2.3% sodium dodecyl sulfate [SDS], 2% beta-mercaptoethanol, 0.005% bromphenol blue pH 6.8), and heated at 95°C for 5 min before separation on a 10% SDS resolving gel with a 4% stacking gel as described by Laemmli (1970). The gel was calibrated with prestained molecular weight markers (Bio-Rad, Richmond, CA), and protein separation was verified by staining with 0.1% Coomassie Blue. For immunoblotting, proteins were electrophoretically transferred to nitrocellulose membranes (Schleicher & Schuell, Keene, NH) using 50 V for 2 h at 4°C as described by Towbin *et al.* (1979). The transfer efficiency was confirmed by Ponceau S staining. Before application of antibody, the nitrocellulose membranes were cut into strips and saturated with 5% nonfat dry milk in PBS (M-PBS) for 1 h at room temperature. Nitrocellulose strips were then incubated for 45 min at room temperature with antibodies diluted 1/100 in 5% M-PBS. Strips were then washed 3 × 30 min with PBS-0.2% Tween 20 to remove unbound antibody. Detection of immunoreactive bands was done by incubation with ^{125}I-protein A (ICN, Costa Mesa, CA) at a specific activity of 0.1 μCi/ml in 5% M-PBS for 45 min at room temperature. Strips were washed for 1 h in PBS-Tween 20 with frequent buffer changes, dried, and exposed to Kodak XAR-5 film (Rochester, NY) overnight.

Probes

Plasmid p59 is a 3.6-kilobase (kb) *Eco*RI/*Bam*HI fragment of lambda ChR-B4 cloned in the vector pBR322. It contains the 3′ end of the 18S rRNA, the 5.8S rRNA, the 5′ end of the 28S rRNA, and the two internal transcribed spacers (Rothblum *et al.*, 1982). The entire nucleotide sequence of this rDNA fragment has been reported (Subrahmanyam *et al.*, 1982; Hadjiolov *et al.*, 1984). The human U3 snRNA probe used was a 4-kb *Hin*dIII/*Eco*RI DNA fragment from clone hU3-1 (Yuan and Reddy, 1989) that contains the entire sequence for U3 snRNA. Probes were labeled by nick translation in the presence of 50 μM bio-11-dUTP (Sigma, St. Louis, MO), dATP, dCTP, and dGTP. The resulting fragments were monitored by electrophoresis in a 1% agarose gel. Nick translation reactions were stopped when a fragment size of ~200 base pairs was obtained. The probe was purified through a G-50 Sephadex (Boehringer Mannheim) column and stored at −20°C.

Northern Analysis

Total RNA was extracted from log growing HeLa cells (Xie and Rothblum, 1991). RNA was denatured in 80% formamide, 10 mM EDTA, 0.5 mg/ml xylene cyanol/bromphenol blue and heated at 65°C for 20 min. Twenty micrograms of RNA per lane was run on 5% polyacrylamide/6 M urea gels in 1× Tris-borate buffer at 150 V. The RNA was transferred in a BioRad electrotransfer apparatus to Zeta Probe (Bio-Rad) nylon membranes in 0.5× TBE at 20 V overnight. After transfer the membranes were baked at 80°C for 2 h. Membranes were prehybridized for 1 h in hybridization buffer (50% formamide, 0.5%

milk powder, 1% SDS, 50 mM phosphate buffer pH 6.5, 0.5 mg/ml denatured and sheared salmon sperm DNA, 10% dextran sulfate). One hundred nanograms of hU3-1 DNA was labeled to a specific radioactivity of 1×10^9 cpm/μg by the random priming method (Sambrook *et al.*, 1989), and unincorporated [^{32}]P dCTP was removed by centrifugation through Sephadex G-25 spin columns. After prehybridization the [^{32}]P-labeled probe was added to a final concentration of 10^6 cpm/ml and hybridized at 42°C for 16 h. After hybridization, membranes were washed four times for a total of 60′. The final 65°C wash contained 0.1× SSC, 1% SDS. The membranes were blotted dry and exposed to X-ray film for 48 h. The size of the hybrid was assessed using single stranded DNA markers.

In Situ Hybridization

Cells were fixed with freshly made 2 or 4% formaldehyde in PBS pH 7.3 for 15 min at room temperature. After rinsing in PBS (3 × 10 min each), cells were permeabilized with 0.2% Triton X-100/0.5% normal goat serum for 5 min at 4°C. Cells were then rinsed in PBS (2 × 10 min each) and then in 2× SSC (1 × 5 min). One hundred nanograms of nick translated probe and 20 μg of competitor *Escherichia coli* tRNA per coverslip were dried down in a Speed Vac (Savant, Farmingdale, NY). Then, 10 μl of deionized formamide was added. The probe and tRNA were denatured by heating for 10 min at 70°C. The probe was chilled on ice immediately, and hybridization buffer containing 4 μl of 50% dextran sulfate, 4 μl of 5% bovine serum albumin (BSA) and 2 μl of 20× SSC was added to each coverslip so that the final concentrations in the hybridization mixture were 5 ng/μl of probe, 1 μg/μl of *E. coli* tRNA, 2× SSC, 1% BSA, and 10% dextran sulfate. Twenty microliters of hybridization mixture was placed onto each coverslip. Coverslips were inverted onto a slide and sealed with rubber cement and incubated in a humid chamber for 16 h at 42°C. After rinsing in 2× SSC/50% formamide at 37°C, 2× SSC and 1× SSC at room temperature for 30 min each, cells were incubated in 4× SSC/0.25% BSA/2 μg/μl avidin-fluorescein isothiocyanate (FITC) (Vector, Burlingame, CA) for 90 min at room temperature in the dark. Coverslips were then rinsed in 4× SSC for 30 min at room temperature, 4× SSC/0.1% Triton X-100, and 4× SSC three times for 10 min each. Cells were stained with 4,6-diamidino-2-phenylindole at a concentration of 1 μg/ml. Coverslips were mounted in 90% glycerol, 10% PBS plus 1 mg/ml of paraphenylenediamine. The final pH was adjusted to 8.0 with 0.5 M carbonate-bicarbonate buffer pH 9.0. Cells were examined with a Nikon (Melville, New York) FXA epifluorescence microscope equipped with a 60×, 1.4 N.A. objective lens.

Other protocols including 4% formaldehyde fixation for 15 min followed by three washes of 10 min each with PBS, dehydration with 50, 70, and 100% ice cold ethanol for 5 min each, air drying and rehydration for 2 min with 2× SSC before hybridization were also used and provided the same results.

In Situ Hybridization-Immunofluorescence Combined Protocol

In situ hybridization followed by immunofluorescence was conducted as described (Jiménez-García and Spector, 1993), using the U3 snRNA or p59 probes to detect the RNAs by FITC-avidin, followed by immunolocalization using antibodies to visualize p80 coilin protein followed by Texas Red conjugated goat-anti-rabbit secondary antibodies (Organon-Teknika).

RESULTS

Localization of rRNA and U3 snRNA During Interphase

Ribosomal RNA and U3 snRNA were localized in HeLa interphase nuclei by fluorescence in situ hybridization. rRNA localized to nucleoli and diffusely throughout the cytoplasm (Figure 1a) using a probe that is complementary to a portion of 18S and 28S rRNAs, 5.8S, and the two internal transcribed spacers (Rothblum *et al.*, 1982). U3 snRNA was observed in nucleoli and in small round bodies that were sometimes associated with the nucleolar surface (Figure 1b, arrows). The distribution, size, and shape of these round bodies that labeled with the U3 snRNA probe were reminisent of previously described coiled bodies (Ramón y Cajal, 1903). To determine if in fact they were coiled bodies, we used in situ hybridization to localize U3 snRNA and immunofluorescence to localize p80 coilin, a protein that is diagnostic

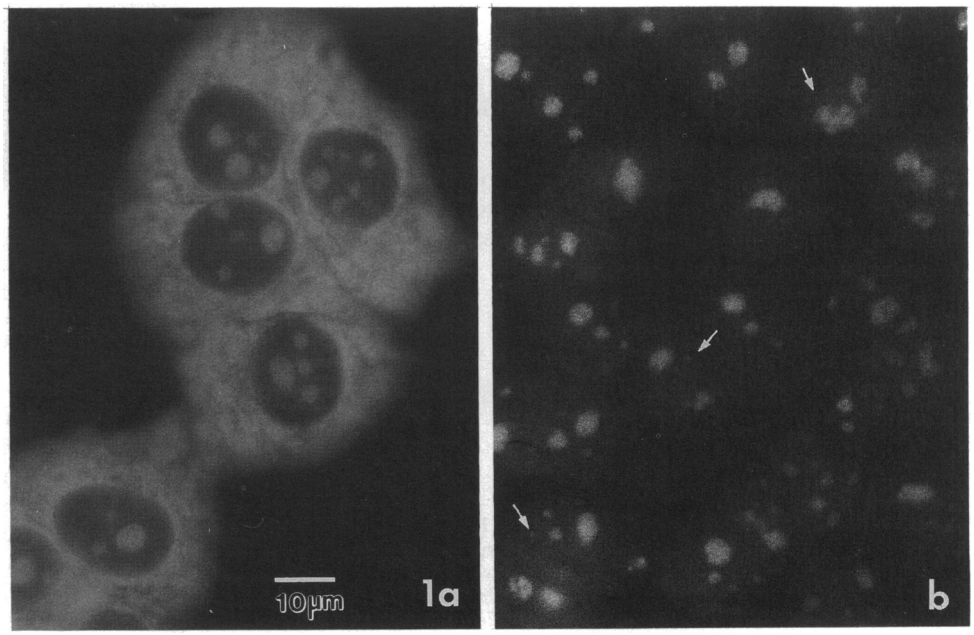

Figure 1. Distribution of rRNA (a) and U3 snRNA (b) in interphase human HeLa cells by fluorescence in situ hybridization. rRNA is localized in the cytoplasm and nucleoli (a). U3 snRNA localizes to nucleoli and also to small round bodies in the nucleoplasm (b, arrows).

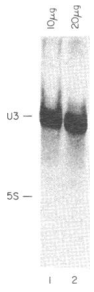

Figure 2. Northern blot analysis of HeLa total RNA with the hU3-1 probe showing hybridization to U3 snRNA.

for coiled bodies (Raska *et al.*, 1991). The specificity of the U3 probe was demonstrated by Northern analysis and the specificity of the coilin antibody was shown by immunoblot. The U3 probe recognized a single band of ~217 nucleotides (Figure 2), and the p80 coilin antibody recognized an 80 kDa protein (Figure 3). We found that the p80 coilin protein colocalized with the round bodies that labeled for U3 snRNA (Figure 4), indicating that these structures were indeed coiled bodies. These double-label studies were performed with FITC- and Texas Red–conjugated antibodies so that the possibility of fluorescence bleedthrough can be ruled out because these two fluorochromes have excitation and emission maxima at different wavelengths. In addition, similar results were obtained when each probe was used independently (unpublished results). The weak signal for U3 snRNA in coiled bodies suggests that either there is a low level of this RNA in coiled bodies or only a small portion of the U3 snRNA is accessible to the probe. Although we did not detect any rRNA in coiled bodies in our initial observations (Figure 1), we still examined the distribution of rRNA and p80 coilin in the same cells and we conclude that coiled bodies do not have any detectable levels of rRNA (Figure 5). Similar results were obtained with a probe complementary to 18S rRNA (unpublished results).

Distribution of rRNA and U3 snRNA During Mitosis in HeLa Cells

Once we knew the localization of rRNA and U3 snRNA in interphase, we were interested in determining the distribution of these RNAs during mitosis. We first examined the fate of the nucleolus during mitosis by NOR silver staining (Figure 6, a–e). During interphase the fibrillar regions of the nucleoli (Figure 6a, arrowhead) and coiled bodies (crossed arrow) are stained by this method. From prophase to anaphase, silver staining is restricted to the NORs (Figure 6, b–d, arrowheads). However, by telophase, silver staining is observed in PNBs (Figure 6e, arrows). We next investigated the dis-

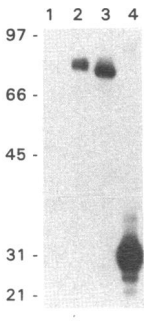

Figure 3. Immunoblotting of HeLa whole cell extracts. Lane 1, normal human serum. Lane 2, rabbit anti-coilin. Lane 3, human anti-coilin. Lane 4, rabbit anti-S6 peptide. Molecular weight markers are designated on the left.

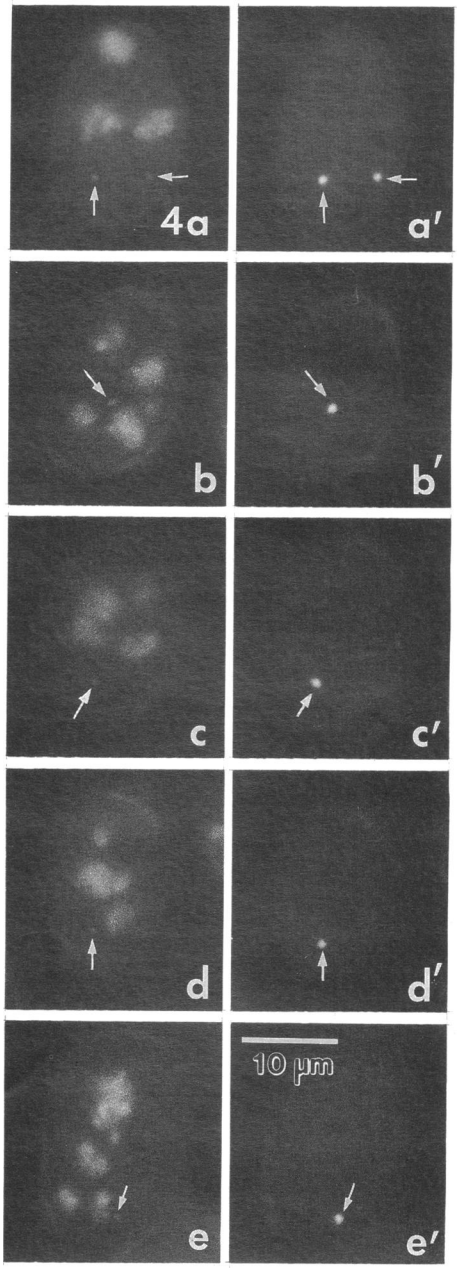

Figure 4. Colocalization of U3 snRNA (a–e) and coilin (a'–e') in interphase HeLa cells by fluorescence in situ hybridization-immunofluorescence combined protocol. U3 snRNA is localized in nucleoli (a–e) and in small round nuclear bodies that are sometimes closely associated with the nucleolus and label with anti-coilin antibodies (a'–e', arrows) demonstrating that these structures are coiled bodies.

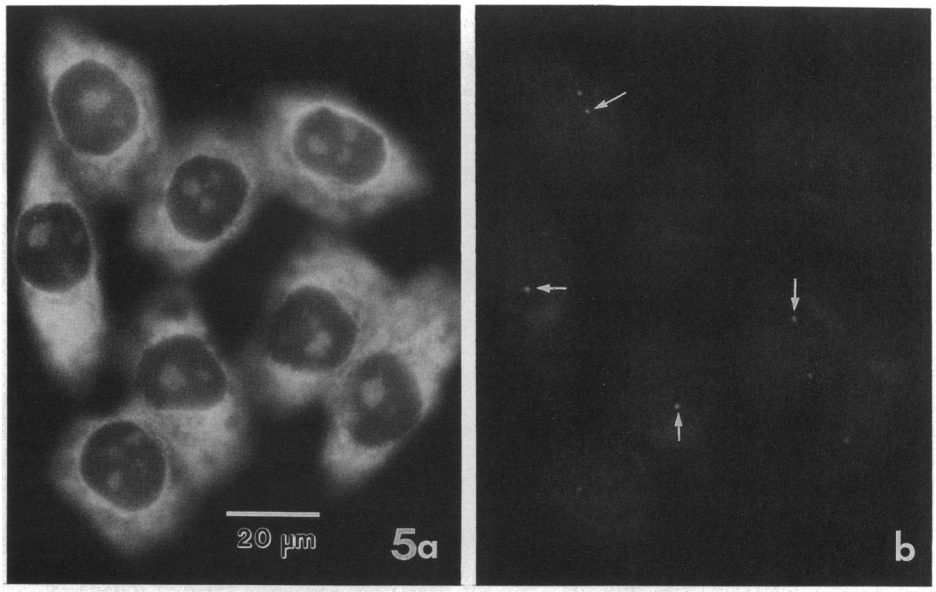

Figure 5. Localization of rRNA (a) and coilin (b) in the same interphase HeLa cells by fluorescence in situ hybridization-immunofluorescence combined protocol. rRNA is observed in nucleoli and in the cytoplasm (a). Coilin localizes in coiled bodies (b, arrows) in the same cells but is not present in regions where rRNA is observed.

tribution of rRNA (Figure 6, f–j) during mitosis. During interphase rRNA was present in the cytoplasm and in nucleoli (Figure 6f), although some nucleolar areas, which may correspond to fibrillar centers or nucleolar vacuoles (Figure 6f, arrowhead), remained unstained. From prophase to anaphase, rRNA was observed to be diffusely distributed throughout the cytoplasm but was excluded from the chromosomal arms (Figure 6, g–j).

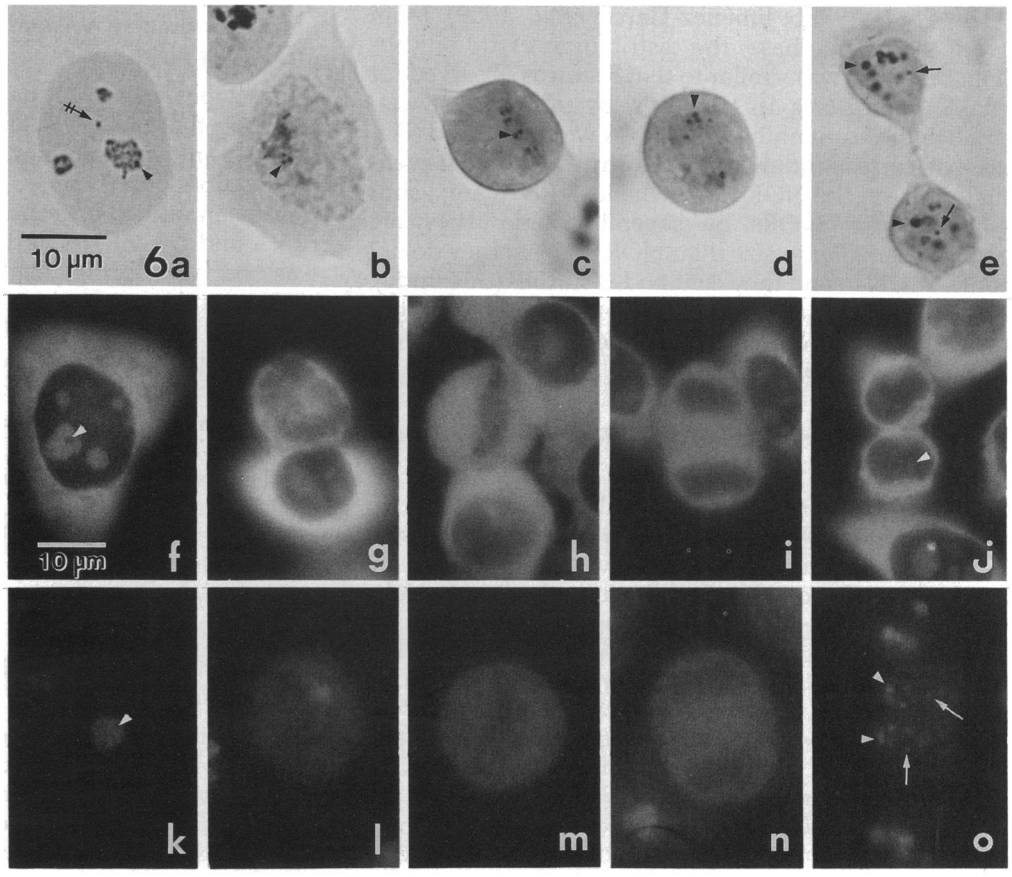

Figure 6. Localization of Ag-NOR proteins by silver staining (a–e) and localization of rRNA (f–j) and U3 snRNA (k–o) by fluorescence in situ hybridization in HeLa cells during mitosis. Silver-staining labels nucleoli (arrowhead) and coiled body (crossed arrow) in interphase (a). During prophase through anaphase (b–d) silver-staining is restricted to NORs (arrowheads). At telophase (e) staining is observed in forming nucleoli (arrowheads) and in prenucleolar bodies (arrows). rRNA is localized to nucleoli in interphase (f) and is observed in the cytoplasm from prophase through anaphase (g–i). During telophase (j) rRNA is detected in the cytoplasm and in the newly forming nucleoli (arrowhead). U3 snRNA is observed in the nucleolus during interphase (k) and it is diffusely distributed in the cytoplasm from prophase to anaphase (l–n). During telophase, U3 snRNA is present in prenucleolar bodies (o, arrows) that will fuse to the NORs (arrowheads). a, f, and k, interphase; b, g, and l, prophase; c, h, and m, metaphase; d, i, and n, anaphase; e, j, and o, telophase.

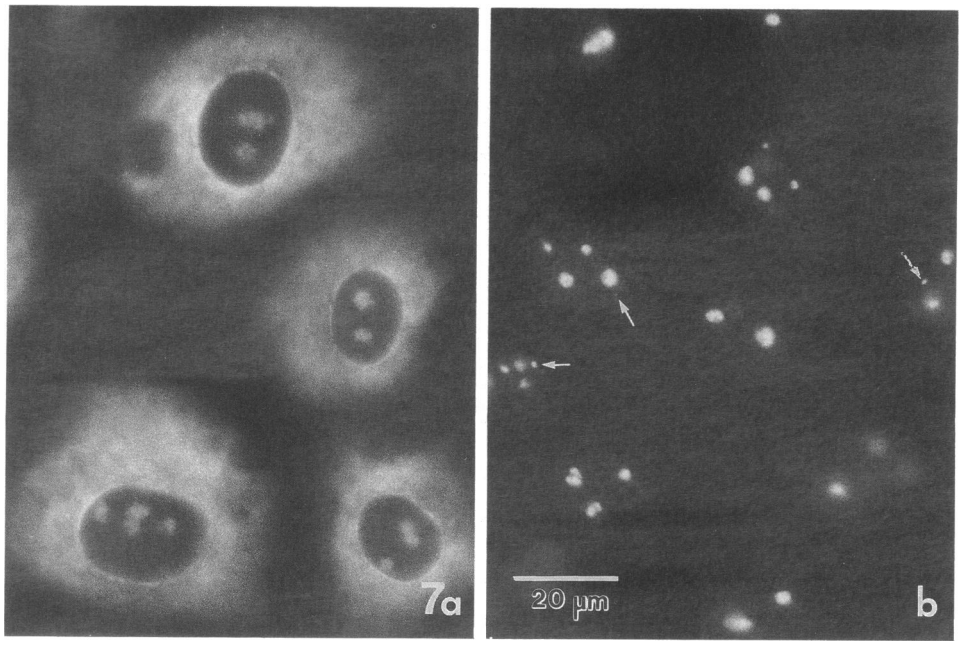

Figure 7. Distribution of rRNA (a) and U3 snRNA (b) in interphase NRK cells by fluorescence in situ hybridization. rRNA is present in nucleoli and in the cytoplasm (a). U3 snRNA is observed in nucleoli and in small round nuclear bodies that in some cases are associated with the nucleoli (b, arrows).

By telophase, rRNA appeared in the newly forming nucleoli (Figure 6j, arrowhead). U3 snRNA was observed in the nucleoli at interphase and appeared to be sublocalized (Figure 6k, arrowhead), similar to what has been reported for the U3-associated protein fibrillarin (Ochs *et al.*, 1985b; Jiménez-García *et al.*, 1989). From prophase to anaphase, the distribution of U3 snRNA (Figure 6, l–n) was similar to that that was observed for rRNA (Figure 6, g–i). However, by telophase several small bodies containing U3 snRNA were observed (Figure 6o), corresponding to PNBs (arrows) that will eventually fuse at the NORs (arrowheads). PNBs are distinguished from NORs by several criteria: they are generally smaller in size than NOR regions, they do not contain rDNA, and they contain U3 snRNP (this study). Nucleolar silver staining and the presence of phosphoprotein C23 are common among both NORs and PNBs (Ochs *et al.*, 1985a).

Distribution of rRNA and U3 snRNA in Interphase NRK Cells

We have previously used NRK cells as a model system to study mitosis (Jiménez-García *et al.*, 1989) because it is a flat cell line that allowed us to visualize PNBs easily at telophase. To corroborate the results obtained in HeLa cells, we repeated the localization of rRNA and U3 snRNA in NRK cells. The same probes were used because rRNA and U3 snRNA are very conserved through evolution (Reddy and Busch, 1988; Yuan and Reddy, 1989). Similar to what was observed in HeLa cells, rRNA was localized in the nucleoli and cytoplasm of NRK cells (Figure 7a), whereas U3 snRNA was present in the nucleoli and other small round bodies (Figure 7b, arrows) that appeared to be coiled bodies. To be sure that these structures were coiled bodies, NRK cells were double-labeled with anti-coilin antibody and the U3 probe. U3 snRNA was present in nucleoli and coiled bodies (Figure 8).

Localization of rRNA and U3 snRNA During Mitosis in NRK Cells

We previously showed that NRK cells contain PNBs at telophase that can be visualized simultaneously by silver staining and by immunofluorescence with antibodies against fibrillarin (Jiménez-García *et al.*, 1989). We performed in situ hybridization on NRK cells to detect rRNA and U3 snRNA during mitosis (Figure 9). rRNA was observed during prophase to anaphase in the cytoplasm and between the chromosome arms but did not localize in association with the condensed chromosomes (Figure 9, b–d). By telophase the only detectable amount of rRNA present in the nucleus was that corresponding to the newly forming nucleolar domains (Figure 9e, arrowheads), namely the NORs. U3 snRNA exhibited a similar distribution to rRNA except during telophase. By telophase, U3 snRNA appeared in numerous PNBs (Figure 9j, arrows) that will fuse to the NORs (arrowheads). These results were identical to those observed in HeLa cells (Figure 6).

Distribution of Ribosomal Protein S6 During Interphase and Mitosis

To test whether any nucleolar factors implicated in ribosome biogenesis are also present in PNBs fusing to the NOR, we examined the localization of ribosomal protein S6 that is a component of the small ribosomal subunit. Rabbit anti-S6 peptide antibody was specific

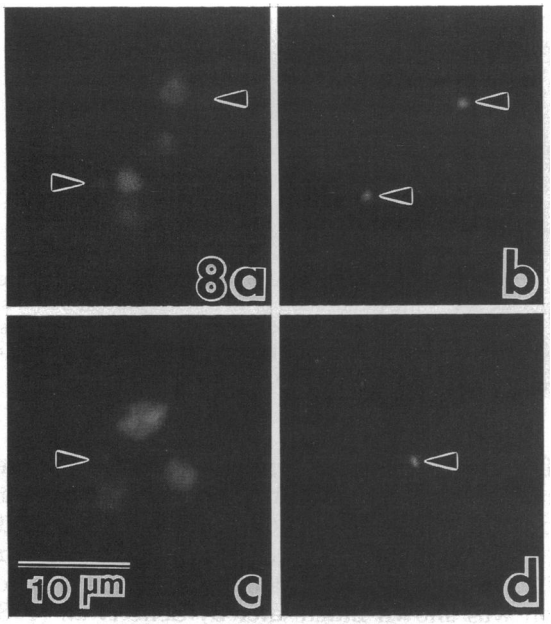

Figure 8. Colocalization of U3 snRNA (a and c) and coilin (b and d) in interphase NRK cells by fluorescence in situ hybridization-immunofluorescence combined protocol. U3 snRNA is localized in nucleoli (a and c) and in small round nuclear bodies that label with anti-coilin antibodies (b and d, arrowheads) demonstrating that these structures are coiled bodies.

for the 31-kDa S6 protein (Collatz *et al.*, 1976), and, just as important, this antibody had no reactivity with the 80-kDa p80-coilin (Figure 3). In interphase, the S6 protein was localized in nucleoli and the cytoplasm, as expected (Figure 10). In addition, it was also present in coiled bodies (Figure 10, arrow heads). However, the absence of 18S rRNA in coiled bodies suggests that the S6 protein is not present in the form of a ribosomal subunit. During mitosis (Figure 11), ribosomal protein S6 shows a similar distribution to rRNA, being restricted to the cytoplasm from prophase (Figure 11b) to anaphase (Figure 11d) and absent from PNBs at telophase (Figure 11e). No detectable amount of S6 protein was observed at the NORs.

DISCUSSION

We have found that U3 snRNA is localized both in nucleoli and coiled bodies during interphase, whereas rRNA was distributed in the nucleoli and throughout the cytoplasm. During nucleologenesis, pre-rRNA was observed in newly forming nucleoli during late telophase but not in PNBs, whereas U3 snRNA was detected in forming nucleoli and PNBs. These results demonstrate that components involved in pre-rRNA processing localize to discrete PNBs at the end of mitosis. Upon reformation of the interphase nucleolus the PNBs, containing factors essential for pre-rRNA processing, are recruited to sites of rRNA transcription and processing (i.e., the NORs).

U3 snRNA and Coiled Bodies

Our finding that U3 snRNA is present in coiled bodies differs from earlier reports by Carmo-Fonseca *et al.* (1992, 1993) who did not find U3 snRNA in these structures. This difference may be because of the fact that the signal for U3 snRNA in the coiled bodies is extremely low compared to the observed signal in nucleoli. In addition, in the present study we used a nick-translated probe containing the entire U3 gene sequence, whereas Carmo-Fonseca and colleagues used an oligonucleotide probe of only 15 bases. Therefore, the number of bio-

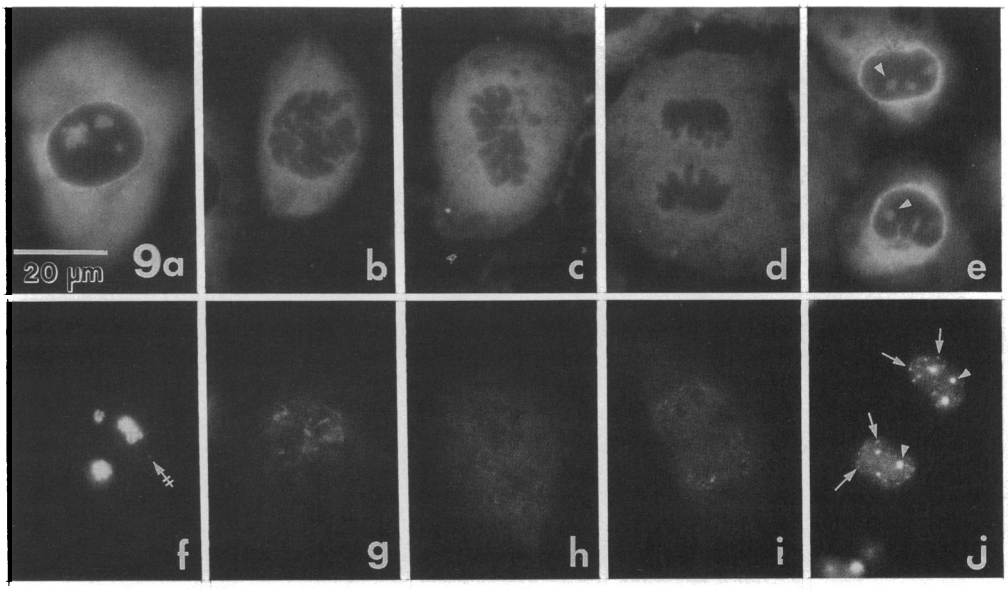

Figure 9. Localization of rRNA (a–e) and U3 snRNA (f–j) in NRK cells during mitosis by fluorescence in situ hybridization. rRNA is present in nucleoli and in the cytoplasm during interphase (a) and is observed diffusely distributed in the cytoplasm during prophase to anaphase (b–d). It appears again in the newly formed and actively synthesizing nucleoli at telophase (e, arrowheads). U3 snRNA is observed in nucleoli and coiled bodies (crossed arrow) in interphase (f) and is distributed in the cytoplasm from prophase through anaphase (g–i). U3 snRNA appears in prenucleolar bodies at telophase (j, arrows) that will fuse to the NORs (arrowheads).

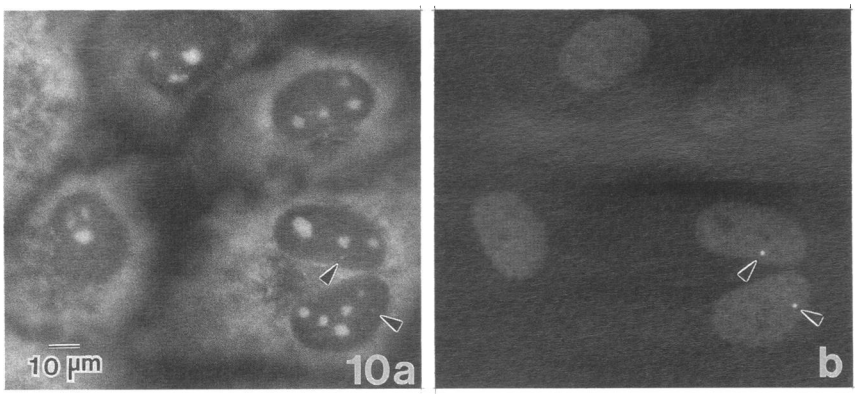

Figure 10. Double-label immunofluorescence of the localization of ribosomal protein S6 (a) and p80-coilin (b). Ribosomal protein S6 localizes to nucleoli, coiled bodies (arrowheads), and the cytoplasm of interphase cells.

tinylated nucleotides (bio-dUTP) available to bind avidin are significantly greater in the present study. We, too, did not observe any labeling using a short oligonucleotide probe complementary to U3 snRNA (provided by Greg Matera, Yale University). However, in a recent report Azum-Gelade (1994) localized U3 snRNA in CHO cells to nucleoli and structures that were presumed to be coiled bodies. In this case the authors used two oligonucleotides corresponding to 43 nucleotides. In a similar type of experiment, Bell *et al.* (1992) did not obtain a positive signal for U3 snRNA in prenucleolar bodies in *Xenopus* egg extracts after a 3-h hybridization for fluorescence in situ hybridization. However, using ^{3}H-labeled antisense probes against *Xenopus* U3, Wu *et al.* (1993) observed hybridization signal after a 12-d exposure. It was suggested by Wu *et al.* (1993) that the rather long exposure required to detect a hybridization signal may be because of the low concentration of U3 snRNA in these bodies. Based on these findings, we urge caution when using oligonucleotide probes to localize sequences that may be present in low copy number at defined spatial positions or when using shorter hybridization or exposure times.

Coiled bodies are intranuclear structures that were observed for the first time by Ramón y Cajal (1903) when using silver impregnation techniques to visualize cells of the nervous system. Ramón y Cajal called these structures accessory bodies of the nucleolus. Sixty-six years later, Monneron and Bernhard (1969) described a small ribonucleoprotein body, which they called a coiled body, in the nucleus of mammalian cells using the EDTA regressive cytochemical technique that preferentially stains ribonucleoproteins at the electron microscopic level (Bernhard, 1969). Subsequently, Hardin *et al.* (1969) suggested that accessory bodies correspond to coiled bodies, although demonstration of this correlation was shown much later by Seite *et al.* (1982) and Lafarga *et al.* (1983). Coiled bodies have been identified in animal and plant cells (Monneron and Bernhard, 1969; Moreno Díaz de la Espina *et al.*, 1982a), although they are more frequently observed in the nuclei of transformed cells (Spector *et al.*, 1992). Recently, a coiled body specific protein, p80 coilin, has been identified (Raska *et al.*, 1990, 1991; Andrade *et al.*, 1991). Partial cDNA sequence analysis has not revealed any sequences that are involved in RNA recognition (Andrade *et al.*, 1991).

It has been suggested that coiled bodies are related to nucleolar function because in their original description they were found in close proximity to the nucleolus (Ramón y Cajal, 1903; Monneron and Bernhard, 1969), because they increase in number when nucleolar transcription is stimulated (Lafarga *et al.*, 1991), and because they stain with the Ag-NOR technique for NORs (Raska *et al.*, 1990). In addition, in cells treated with drugs that induce nucleolar segregation, p80-coilin colocalizes in the segregated nucleolus with fibrillarin (Raska *et al.*, 1990), a 34-kDa protein associated with the nucleolar U3 snRNP particle (Lischwe *et al.*, 1985; Ochs *et al.*, 1985b; Tyc and Steitz, 1989). Perhaps the best demonstration of an association between coiled bodies and nucleoli was reported by Ochs *et al.* (1994), who iden-

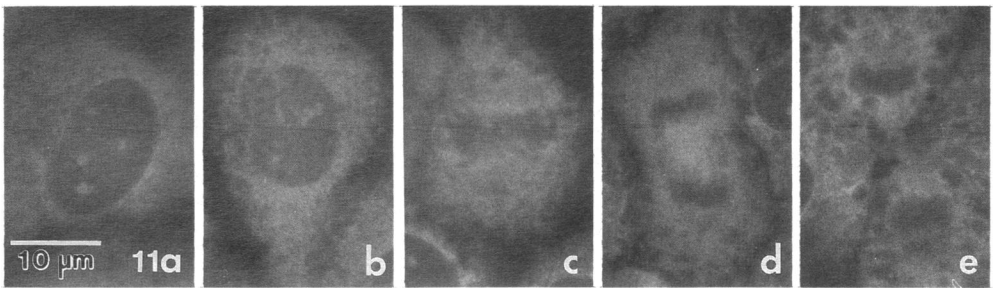

Figure 11. Localization of ribosomal protein S6 in NRK cells during mitosis by immunofluorescence. Ribosomal protein S6 is observed in nucleoli and the cytoplasm at interphase (a). During prophase to telophase it is redistributed throughout the cytoplasm (b–e) with no detectable amounts present in the newly forming nucleoli or in prenucleolar bodies (e).

tified coiled bodies within the nucleoli of certain breast cancer cells. Because coiled bodies contain the nucleolar constituents U3 snRNA, fibrillarin, ribosomal protein S6, and DNA topoisomerase I, but not rRNA, the possibility exists that one of the functions of coiled bodies may be to transport nucleolar components between the nuclear envelope or nucleoplasm and their site of utilization in the nucleolus or vice versa.

Coiled Bodies and Pre-mRNA Processing

In addition to the aforementioned nucleolar association, coiled bodies also contain snRNPs (Fakan *et al.*, 1984; Carmo-Fonseca *et al.*, 1991a,b; Raska *et al.*, 1991; Spector *et al.*, 1992) that are involved in pre-mRNA processing. Based on this, coiled bodies have been suggested to have a potential role in pre-mRNA metabolism (Carmo-Fonseca *et al.*, 1991a,b). However, because they do not contain other pre-mRNA processing components such as heterogeneous nuclear RNPs (Carmo-Fonseca *et al.*, 1991a; Raska *et al.*, 1991) and the non-snRNP splicing factor SC35 (Carmo-Fonseca *et al.*, 1991; Spector *et al.*, 1991; Huang and Spector, 1992), it is unlikely that they play an essential role in pre-mRNA processing. Moreover, coiled bodies do not contain DNA (Monneron and Bernhard, 1969; Martín *et al.*, 1992), and ^{3}H-uridine incorporation does not occur within these structures (Fakan and Bernhard, 1971; Fakan *et al.*, 1976; Moreno Díaz de la Espina *et al.*, 1982a). These findings make it difficult to suggest an essential role for coiled bodies in pre-mRNA splicing.

Recently, a coilin-related protein was identified in C-snurposomes (Tuma et al., 1993). In addition, Wu *et al.* (1993) showed the staining of C-snurposomes with a polyclonal antibody (Wu *et al.*, 1993) that recognizes a coilin fusion protein (Andrade *et al.*, 1993). These studies suggest a possible relationship between C-snurposomes found in the germinal vesicle of Amphibian oocytes and coiled bodies, thus far identified only in mammalian and plant cells. In addition to a coilin homologue, U7 snRNA, which functions in 3′ end processing of histone pre-mRNAs (Mowry and Steitz, 1987), was also localized in C-snurposomes (Wu and Gall, 1993). The presence of U7 snRNA and coilin reactivity in C-snurposomes, some of which are associated with the histone loci on chromosomes in germinal vesicles (Gall *et al.*, 1981), suggests a potential role for coiled bodies in histone 3′ end processing.

U3 snRNA and Prenucleolar Bodies

Although rRNA and U3 snRNA are both uniformly distributed throughout the cytoplasm of mitotic cells, they remain absent from the chromosomes during mitosis, only U3 snRNA appears in PNBs during telophase. Our data confirms a recent finding by Azum-Gelade *et al.* (1994) that reported the presence of U3 snRNA in prenucleolar bodies at the end of telophase in CHO cells. PNBs are fibrillar structures (Stevens, 1965) of RNP nature (Risueño and Medina, 1986) that form from material surrounding chromosomes and that coalesce during telophase by fusing at the NOR. Formation of PNBs has been shown to be independent of transcriptional activity (Morcillo *et al.*, 1976; Benavente *et al.*, 1987) or the presence of rDNA (Bell *et al.*, 1992). However, fusion of PNBs to the NOR does depend upon transcriptional activity of RNA polymerase I (Morcillo *et al.*, 1976; Ochs *et al.*, 1985a; Bell *et al.*, 1992). Coincident with transcriptional activation of RNA polymerase I at the NORs, PNBs fuse to the NORs and provide the essential factors (i.e., U3 snRNP) necessary for the processing of pre-rRNA and the formation of a functional nucleolus (Goessens, 1984; Ochs *et al.*, 1985a). Because U-snRNAs are of long half-life (Reddy and Busch, 1988), our results suggest that the fidelity of these inherited RNAs is crucial for the initial pre-rRNA processing events that occur in the newly forming nucleoli at telophase. The availability of pre-existing U3 snRNPs assures that any preexisting (Fan and Penman, 1971) and newly synthesized transcripts are rapidly processed in anticipation of the high rate of protein synthesis characteristic of the G1 phase of the cell cycle.

Nucleolar Formation and RNA Polymerase I Transcription

In addition to the present finding of U3 snRNA in PNBs, we have previously shown that fibrillarin, the major protein in the U3 snRNP, also appears in prenucleolar bodies during telophase (Ochs *et al.*, 1985b; Jiménez-García *et al.*, 1989). Additionally, proteins B23 and C23 also localize to PNBs (Ochs *et al.*, 1983, 1985a), suggesting that molecules implicated in transcription and processing of pre-rRNA are segregated to daughter cells during mitosis or are synthesized very early on after anaphase. However, ribosomal proteins S1 (Bell *et al.*, 1992) and S6 (this report) are not present in PNBs, indicating that some ribosomal proteins do not arrive at the newly forming nucleolus by PNB-mediated fusion. Hence, in the formation of the nucleolus, loci containing rDNA (NORs) are indeed the sites that "organize" or recruit preexisting nucleolar material to form the nucleolus. Further support that active transcription is necessary for nucleolar formation was demonstrated in *Xenopus* egg extracts (Bell *et al.*, 1992) that lacked functional NORs. Bell *et al.* (1992) observed the assembly of prenucleolar bodies in the extracts but not the fusion of these bodies into a nucleolus. In addition, if rRNA synthesis is inhibited in situ during telophase, the nucleolus does not form (Semeshin *et al.*, 1975) after mitosis. The partial formation of the nucleolus in the presence of actinomycin D or after microinjection of antibodies against RNA polymerase I (Benavente *et al.*, 1987) suggests that some incompletely transcribed or unprocessed pre-rRNA has remained present during mitosis, so these rRNAs would presumably contribute to the partial assembly of the nucleolus. In fact, it is

known that pre-rRNA persists in dividing cells and starts to mature during telophase (Fan and Penman, 1971). A probe specific for the initial transcribed segment of the external transcribed spacer should help in detecting these partially transcribed (engaged) molecules, because the probe used here (p59) only recognizes part of the 18S, the two internal transcribed spacers, 5.8S and part of 28S rRNAs.

In summary, we have demonstrated that during nucleologenesis the U3 snRNA that is involved in pre-rRNA processing is contained in PNBs and newly forming nucleoli at the NORs, whereas rRNA is observed only at the NORs. Because fusion of PNBs is dependent upon pre-rRNA transcription, these results suggest that coupling of transcription and processing is coordinated by a recruiting mechanism originating as a consequence of transcriptional activity. We propose a general model in which factors involved in RNA processing, both pre-mRNA (Jiménez-García and Spector, 1993) and pre-rRNA, are recruited to sites of active transcription from storage and/or assembly sites.

ACKNOWLEDGMENTS

We thank Dr. R. Reddy (Baylor College of Medicine, Houston, TX) for plasmid hU3-1 and Dr. R. Traut (University of California, Davis, CA) for the gift of antibody to ribosomal protein S6. This work was supported in part by Dirección General de Asuntos del Personal Académico, U.N.A.M., Mexico, through a postdoctoral fellowship to L.F.J.-G. and grants from the National Institutes of Health (GM-42694 and 5P30CA-45508-06) to D.L.S.

REFERENCES

Andrade, L.E.C., Chan, E.K.L., Raska, I., Peebles, C.L., Roos, G., and Tan, E.M. (1991). Human autoantibody to a novel protein of the nuclear coiled body: immunological characterization and cDNA cloning of p80-coilin. J. Exp. Med. *173*, 1407–1419.

Andrade, L.E.C., Tan, E.M., and Chan, E.K.L. (1993). Immunocytochemical analysis of the coiled body in the cell cycle and during cell proliferation. Proc. Natl. Acad. Sci. USA *90*, 1947–1951.

Azum-Gélade, M.-C., Noaillac-Depeyre, J., Caizergues-Ferrer, M., and Gas, N. (1994). Cell cycle redistribution of U3 snRNA and fibrillarin. Presence in the cytoplasmic nucleolus remnant and in the prenucleolar bodies at telophase. J. Cell. Sci. *107*, 463–475.

Bell, P., Dabauvalle, M.-C., and Scheer, U. (1992). In vitro assembly of prenucleolar bodies in *Xenopus* egg extract. J. Cell Biol. *118*, 1297–1304.

Benavente, R., Rose, K.M., Reimer, G., Hagle-Derr, B., and Scheer, U. (1987). Inhibition of nucleolar reformation after microinjection of antibodies to RNA polymerase I into mitotic cells. J. Cell Biol. *105*, 1483–1491.

Bernhard, W. (1969). A new procedure for electron microscopical cytology. J. Ultrastruct. Res. *27*, 250–265.

Busch, H., and Smetana, K. (1970). The Nucleolus, New York: Academic Press.

Carmo-Fonseca, M., Ferreira, J., and Lamond, A.I. (1993). Assembly of snRNP-containing coiled bodies is regulated in interphase and mitosis. Evidence that the coiled body is a kinetic nuclear structure. J. Cell Biol. *120*, 841–852.

Carmo-Fonseca, M., Pepperkok, R., Carvalho, M.T., and Lamond, A.I. (1992). Transcription-dependent colocalization of the U1, U2, U4/U6 and U5 snRNPs in coiled bodies. J. Cell Biol. *117*, 1–14.

Carmo-Fonseca, M., Pepperkok, R., Sproat, B.S., Ansorge, W., Swanson, M., and Lamond, A.I. (1991a). In vivo detection of snRNP-rich organelles in the nuclei of mammalian cells. EMBO J. *10*, 1863–1873.

Carmo-Fonseca, M., Tollervey, D., Barabino, S.M.L., Merdes, A., Brunner, C., Zamore, P.D., Green, M.R., Hurt, E., and Lamond, A.I. (1991b). Mammalian nuclei contain foci which are highly enriched in components of the pre-mRNA splicing machinery. EMBO J. *10*, 195–206.

Chan, E.K.L., Imai, H., Hamel, J.C., and Tan, E.M. (1991). Human autoantibody to RNA polymerase I transcription factor hUBF. Molecular identity of nucleolus organizer region autoantigen NOR-90 and ribosomal RNA upstream binding factor. J. Exp. Med. *174*, 1239–1244.

Chan, E.K.L., and Pollard, K.M. (1992). Autoantibodies to ribonucleoprotein particles by immunoblotting. In: Manual of Clinical Laboratory Immunology, 4th ed., eds. N.R. Rose, E.C. deMacario, J.L. Fahey, H. Friedman, and G.M. Penn, Washington, DC: American Society of Microbiology, 755–762.

Chan, Y.-L., and Wool, I.G. (1988). The primary structure of rat ribosomal protein S6. J. Biol. Chem. *263*, 2891–2896.

Collatz, E., Wool, I.G., Lin, A., and Stoffler, G. (1976). The isolation of eukaryotic ribosomal proteins. J. Biol. Chem. *251*, 4666–4672.

De la Torre, C., Fernandez-Gomez, M.E., and Giménez-Martín, G. (1975). Rate of nucleologenesis as a measure of gene activity. Nature *256*, 503–505.

De la Torre, C., and Giménez-Martín, G. (1982). The nucleolar cycle. In: The Nucleolus, eds. E.G. Jordan and C.A. Cullis, Cambridge, UK: Cambridge University Press, 153–177.

Fakan, S., and Bernhard, W. (1971). Localization of rapidly and slowly labeled nuclear RNA as visualized by high resolution autoradiography. Exp. Cell Res. *67*, 129–141.

Fakan, S., Leser, G., and Martin, T.E. (1984). Ultrastructural distribution of nuclear ribonucleoproteins as visualized by immunocytochemistry in thin sections. J. Cell Biol. *98*, 358–363.

Fakan, S., Puvion, E., and Spohr, G. (1976). Localization and characterization of newly synthesized nuclear RNA in isolated rat hepatocytes. Exp. Cell Res. *99*, 155–164.

Fan, H., and Penman, S. (1971). Regulation of synthesis and processing of nucleolar components in metaphase arrested cells. J. Mol. Biol. *59*, 27–42.

Gall, J.G., Stephenson, E.C., Erba, H.P., Diaz, M.O., and Barsacchi-Pilone, G. (1981). Histone genes are located at the sphere loci of newt lampbrush chromosomes. Chromosoma *84*, 159–171.

Goessens, G. (1984). Nucleolar structure. Int. Rev. Cytol. *87*, 107–158.

Goodpasture, C., and Bloom, S.E. (1975). Visualization of nucleolar organizer regions in mammalian chromosomes using silver staining. Chromosoma *53*, 37–50.

Hadjiolov, A.A. (1985). The nucleolus and ribosome biogenesis. In: Cell Biology Monographs, vol. 12, New York: Springer-Verlag, 1–263.

Hadjiolov, A.A., Georgiev, O.I., Nosikov, V.V., and Yavachev, L.P. (1984). Primary and secondary structure of rat 28S ribosomal RNA. Nucleic Acids Res. *124*, 3677–3693.

Hardin, J.H., Spicer, S.S., and Greene, W.B. (1969). The paranucleolar structure, accessory body of Cajal, sex chromatin, and related structures in nuclei of rat trigeminal neurons: a cytochemical and ultrastructural study. Anat. Rec. *164*, 403–432.

Huang, S., and Spector, D.L. (1992). U1 and U2 snRNAs are present in nuclear speckles. Proc. Natl. Acad. Sci. USA *89*, 305–308.

Hughes, J.M., and Ares, M., Jr. (1991). Depletion of U3 small nucleolar RNA inhibits cleavage in the 5′ external transcribed spacer of yeast pre-ribosomal RNA and impairs formation of 18S ribosomal RNA. EMBO J. *10*, 4231–4239.

Jiménez-García, L.F., Rothblum, L.I., Busch, H., and Ochs, R.L. (1989). Nucleologenesis: use of non-isotopic in situ hybridization and immunocytochemistry to compare the localization of rDNA and nucleolar proteins during mitosis. Biol. Cell *65*, 239–246.

Jiménez-García, L.F., and Spector, D.L. (1993). In vivo evidence that transcription and pre-mRNA processing are coordinated by a recruiting mechanism. Cell *73*, 47–59.

Kass, S., Tyc, K., Steitz, J.A., and Sollner-Webb, B. (1990). The U3 small nucleolar ribonucleoprotein functions in the first step of preribosomal RNA processing. Cell *60*, 897–908.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature *227*, 680–685.

Lafarga, M., Andrés, M.A., Berciano, M.T., and Maquiera, E. (1991). Organization of nucleoli and nuclear bodies in osmotically stimulated supraoptic neurons of the rat. J. Comp. Neurol. *308*, 329–339.

Lafarga, M., Hervás, J.P., Santa-Cruz, M.C., Villegas, J., and Crespo, D. (1983). The "accessory body" of Cajal in the neuronal nucleus. A light and electron microscopic approach. Anat. Embryol. *166*, 19–30.

Lafontaine, J.G. (1958). Structure and mode of formation of the nucleolus in meristematic cells of *Vicia faba* and *Allium cepa*. J. Biophys. Biochem. Cytol. *4*, 777–784.

Lafontaine, J.G., and Chouinard, L.A. (1963). A correlated light and electron microscope study of the nucleolar material during mitosis in *Vicia faba*. J. Cell Biol. *17*, 167–201.

Lischwe, M.A., Ochs, R.L., Reddy, R., Cook, R.G., Yeoman, L.C., Tan, E.M., Reichlin, M., and Busch, H. (1985). Purification and partial characterization of a nucleolar scleroderma antigen (Mr = 34,000; pI 8.5) rich in N^G, N^G-dimethylarginine. J. Biol. Chem. *260*, 14304–14310.

Martín, M., Moreno Díaz de la Espina, S., Jiménez-García, L.F., Fernandez-Gomez, M.E., and Medina, F.J. (1992). Further investigations on the functional role of two nuclear bodies in onion cells. Protoplasma *167*, 175–182.

McClintock, B. (1934). The relation of a particular chromosomal element to the development of the nucleoli in *Zea mays*. Z. Zellforsch. Mikrosk. Anat. *21*, 294–328.

Monneron, A., and Bernhard, W. (1969). Fine structural organization of the interphase cell nucleus of some mammalian cells. J. Ultrastruct. Res. *27*, 266–288.

Montgomery, T.H., Jr. (1898). Comparative cytological studies, with especial regard to the morphology of the nucleolus. J. Morphol. *15*, 265–584.

Morcillo, G., de la Torre, C., and Giménez-Martín, G. (1976). Nucleolar transcription during plant mitosis. Exp. Cell Res. *102*, 311–316.

Moreno Díaz de la Espina, S., Risueño, M.C., and Medina, F.J. (1982a). Ultrastructural, cytochemical and autoradiographic characterization of coiled bodies in the plant cell nucleus. Biol. Cell *44*, 229–238.

Moreno Díaz de la Espina, S., Sanchez-Pina, M.A., and Risueño, M.C. (1982b). Localization of acid phosphatase activity, phosphate ions and inorganic cations in plant nuclear coiled bodies. Cell Biol. Int. Rep. *6*, 601–607.

Mowry, K.L., and Steitz, J.A. (1987). Identification of the human U7 snRNP as one of several factors in the 3′ end maturation of histone premessenger RNAs. Science *238*, 1682–1687.

Ochs, R.L., Lischwe, M., O'Leary, P., and Busch, H. (1983). Localization of nucleolar phosphoproteins B23 and C23 during mitosis. Exp. Cell Res. *146*, 139–149.

Ochs, R.L., Lischwe, M.A., Shen, E., Carrol, R.E., and Busch, H. (1985a). Nucleologenesis: composition and fate of prenucleolar bodies. Chromosoma *92*, 330–336.

Ochs, R.L., Lischwe, M.A., Spohn, W.H., and Busch, H. (1985b). Fibrillarin: a new protein of the nucleolus identified by autoimmune sera. Biol. Cell *54*, 123–134.

Ochs, R.L., Stein, T.W., Jr., and Tan, E.M. (1994). Coiled bodies in the nucleolus of breast cancer cells. J. Cell Sci. *107*, 385–399.

Peculis, B., and Steitz, J.A. (1993). Disruption of U8 nucleolar snRNA inhibits 5.8S and 28S rRNA processing in the *Xenopus* oocyte. Cell *73*, 1233–1245.

Perry, R.P. (1976). Processing of RNA. Annu. Rev. Genet. *45*, 605–639.

Pfeifle, J., Boller, K., and Anderer, F.A. (1986). Phosphoprotein pp135 is an essential component of the nucleolus organizer region (NOR). Exp. Cell Res. *162*, 11–22.

Ploton, D., Thiry, M., Menager, M., Lepoint, A., Adnet, J.J., and Goessens, G. (1987). Behavior of the nucleolus during mitosis. Chromosoma *100*, 95–107.

Puvion-Dutilleul, F., Mazan, S., Nicoloso, M., Christensen, M.E., and Bachellerie, J.-P. (1991). Localization of U3 RNA molecules in nucleoli of HeLa and mouse 3T3 cells by high resolution in situ hybridization. Eur. J. Cell Biol. *56*, 178–186.

Puvion-Dutilleul, F., Mazan, S., Nicoloso, M., Pichard, E., Bachellerie, J.-P., and Puvion, E. (1992). Alterations of nucleolar ultrastructure and ribosome biogenesis by actinomycin D. Implications for U3 snRNP function. Eur. J. Cell Biol. *58*, 149–162.

Ramón y Cajal, S. (1903). Un sencillo método de coloracion del retículo protoplasmico y sus efectos en los diversos órganos nerviosos. Trab. Lab. Invest. Biol. *2*, 129–221.

Raska, I., Andrade, L.E.C., Ochs, R.L., Chan, E.K.L., Roos, G., and Tan, E.M. (1991). Immunological and ultrastructural studies of the nuclear coiled body with autoimmune antibodies. Exp. Cell Res. *195*, 27–37.

Raska, I., Ochs, R.L., Andrade, L.E.C., Chan, E.K.L., Burlingame, R., Peebles, C., Gruol, D., and Tan, E.M. (1990). Association between the nucleolus and the coiled body. J. Struct. Biol. *104*, 120–127.

Reddy, R., and Busch, H. (1988). Small nuclear RNAs: RNA sequence, structure and modifications. In: Structure and Function of Major and Minor Small Nuclear Ribonucleoprotein Particles, ed. M.L. Birnstiel, New York: Springer-Verlag, 1–37.

Risueño, M.C., and Medina, F.J. (1986). The nucleolar structure in plant cells. Servicio Editorial Universidad del País Vasco, vol. 7, Vizcaya, Spain.

Ritossa, F.M., and Spiegelman, S. (1965). Localization of DNA complementary to ribosomal RNA in the nucleolus organizer region of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA *53*, 737–745.

Rothblum, L.I., Parker, D.L., and Cassidy, D. (1982). Isolation and characterization of rat ribosomal DNA clones. Gene *172*, 75–77.

Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989). Molecular Cloning, A Laboratory Manual, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Savino, R., and Gerbi, S.A. (1990). In vivo disruption of *Xenopus* U3 snRNA affects ribosomal RNA processing. EMBO J. *9*, 2299–2308.

Scheer, U., and Benavente, R. (1990). Functional and dynamic aspects of the mammalian nucleolus. BioEssays *12*, 14–21.

Scheer, U., and Rose, K.M. (1984). Localization of RNA polymerase I in interphase cells and mitotic chromosomes by light and electron microscopic immunocytochemistry. Proc. Natl. Acad. Sci. USA *81*, 1431–1435.

Schmidt-Zachmann, M., Hàgle-Dèrr, B., and Franke, W.W. (1987). A constitutive nucleolar protein identified as a member of the nucleoplasmin family. EMBO J. *6*, 1881–1890.

Seite, R., Pebusque, M.J., and Vio-Cigna, M. (1982). Argyrophilic proteins on coiled bodies in sympathetic neurons identified by Ag-NOR procedure. Biol. Cell *46*, 97–100.

Semeshin, V.F., Sherodilo, A.I., and Beyaeva, E.S. (1975). Nucleolar formation under inhibited RNA synthesis. Exp. Cell Res. *93*, 458–467.

Spector, D.L., Fu, X.-D., and Maniatis, T. (1991). Associations between distinct pre-mRNA splicing components and the cell nucleus. EMBO J. *10*, 3467–3481.

Spector, D.L., Lark, G., and Huang, S. (1992). Differences in snRNP localization between transformed and nontransformed cells. Mol. Biol. Cell *3*, 555–569.

Spector, D.L., and Smith, H.C. (1986). Redistribution of U-snRNPs during mitosis. Exp. Cell Res. *163*, 87–94.

Stevens, B. (1965). The fine structure of the nucleolus during mitosis in the grasshopper neuroblast cell. J. Cell Biol. *24*, 349–368.

Stockert, J.C., Fernandez-Gomez, M.E., Giménez-Martín, G., and Sanchez-Lopez, J.F. (1970). Organization of argyrophilic nucleolar material throughout the division cycle of meristematic cells. Protoplasma *69*, 265–278.

Subrahmanyam, C.S., Cassidy, B., Busch, H., and Rothblum, L.I. (1982). Nucleotide sequence of the region between the 18S rRNA sequence and the 28S rRNA sequence of rat ribosomal DNA. Nucleic Acids Res. *10*, 3667–3680.

Towbin, H., Staehelin, T., and Gordon, J. (1979). Electrophoretic transfer of protein from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA *76*, 4350–4354.

Tuma, R.S., Stolk, J.A., and Roth, M.B. (1993). Identification and characterization of a sphere organelle protein. J. Cell Biol. *122*, 767–773.

Tyc, K., and Steitz, J.A. (1989). U3 and U13 comprise a new class of mammalian snRNPs localized in the cell nucleolus. EMBO J. *8*, 3113–3119.

Yuan, Y., and Reddy, R. (1989). Genes for human U3 small nucleolar RNA contain highly conserved flanking sequences. Biochim. Biophys. Acta. *1008*, 14–22.

Xie, W.-Q., and Rothblum, L. (1991). Rapid small scale RNA isolation from tissue culture cells. Biotechniques *12*, 1266–1275.

Wu, C.-H.H., and Gall, J.G. (1993). U7 small nuclear RNA in C-snurposomes of the *Xenopus* germinal vesicle. Proc. Natl. Acad. Sci. USA *90*, 6257–6259.

Wu, Z., Murphy, C., Wu, C.-H., Tsvetkov, A., and Gall, J.G. (1993). Snurposomes and coiled bodies. Cold Spring Harbor Symp. Quant. Biol. *58*, 747–754.